IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 030523/0185

In re patent application of
VELANDER *et al.*

Group Art Unit: 1632

Serial No. 10/062,447

Examiner: D. Crouch

Filed: February 5, 2002

For: EXPRESSION OF ACTIVE FACTOR IX IN MAMMARY TISSUE OF TRANSGENIC ANIMALS

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

I, William H. Velander, hereby declare:

1. I am an inventor of the captioned application. I have worked in the field of transgenic animals and expressing blood proteins from transgenic animals since 1987. I have published over 45 papers with a number of these papers in the transgenic animal field, and I am an inventor of U.S. Patent Nos. 5,589,604; 5,831,141; 5,880,327; 6,255,554; 6,262,336; 6,344,596 and 6,518,482, and their corresponding foreign applications, all of which are directed to subject matter in the transgenic animal field. I received a Ph.D. in Chemical Engineering in 1987 from the Pennsylvania State University, and joined the Department of Chemical Engineering at the Virginia Polytechnic Institute and State University in 1986. Since 2003, I have held the position as the Chair of the Department of Chemical Engineering at the University of Nebraska-Lincoln. Attached is my CV to further explain my experience and background.

2. I have read and understood the Office Action dated March 15, 2004, and particularly the Examiner's comments regarding the alleged anticipation or alternatively obviousness of the claimed Factor IX and its use to treat hemophilia B by the disclosure in Kim *et al*, (hereinafter referred to as "Kim"), cited by the Examiner in the Office Action, which discloses treatment of hemophilia B by the administration of human plasma-derived monoclonal antibody purified Factor IX.

3. In response to the Examiner's comments on page 2 of the Office Action that "[w]hile it is recognized that the claims require that the factor IX be from a transgenic nonhuman mammal, there are no characteristics of the Factor IX of the claimed method that distinguish it from the Factor IX of Kim," I provide the following information to show that the claimed biologically active Factor IX that is expressed in transgenic pigs possesses properties that are different from the plasma-derived monoclonal antibody purified Factor IX disclosed in Kim.

4. My research group has performed comparative studies of the biological activity of the monoclonal antibody-purified factor IX concentrate (commercially known as Mononine®), the same Factor IX preparation disclosed and studied in Kim, with the Factor IX produced in transgenic pigs (hereinafter referred to as "TG FIX"). Biological activity was measured by following the clotting activity as a function of time for the intravenously infused Factor IXs. In these experiments, both of these Factor IXs were infused intravenously into hemophilia B mice (n=8 in each group) at concentrations up to 300 U per kg. Hemophilia B mice are known to be a reliable preclinical model for the assessment of biological activity of Factor IX in humans. Factor IX activity and antigen levels recovered at 15 minutes post infusion ranged from 34-65% for Mononine® and 22-26% for TG FIX. Shown in attached Figure 1 (See Attachment B) is a semi-log plot of Factor IX activity versus time for one representative set of experiments comparing TG FIX and Mononine® (% Plasma Activity, where 100% = 1 IU/ml). While Mononine® had a higher post-infusion recovery of activity and antigen, the decay of Mononine® in the mouse was faster than for the TG FIX. Both Factor IX products were able to sustain about 5% of normal Factor IX activity (0.05 IU/ml) in plasma for 72 hours post-infusion. A level of 5% or more is considered sufficient to render normal blood clotting.

5. Calculation of the half-life and mean residence time (MRT) from the above described data shown in Figure 1 is useful for characterizing the biological activity of different Factor IXs. Both the half-life and MRT values are non-linear descriptions of the biologically activity of Factor IX in circulation. Furthermore, the longevity of the clotting activity of the Factor IXs in circulation is frequently described by the determination of the MRT or the half-life in the post recovery phase, i.e., the first 15 minutes post-infusion of Factor IX. From our studies of the

biological activity of both TG FIX and Mononine® in hemophilia B mice, we determined both the half-life and MRT. The half-life of TG FIX is approximately 27 hours in circulation whereas the half-life of Mononine® is approximately only 17 hours. The MRT of TG FIX is approximately 40 hours whereas the MRT of Mononine® is approximately 20 hours. These relative measurements evaluate the biological activity of the Factor IXs in a manner that includes all of its biological properties to include the effectiveness of the clotting activity in circulation.

6. These determinations provide evidence that the TG FIX of the present invention possesses a different *in* vivo recovery, half-life, and MRT as compared to Mononine® as described in Kim. From this evidence, it is my opinion that TG FIX behaves differently in circulation by proportioning itself to the vascular endothelial cells and into the extravascular spaces differently than Mononine®. Thus, I conclude from these differences in biological activity that the claimed TG FIX is different from Mononine® as disclosed in Kim, and therefore, the method of treatment also is different.

7. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____
William H. Velander, Ph.D.

Date: August 8, 2004
or August eighth 2004

Biosketch and Curriculum Vita of William H. Velander

Dr. Velander has been working on safer, abundant sources of plasma-derived medicines since 1987, when disease contamination of blood supply medicines by HIV, Hepatitis B and C reached a world-wide epidemic. To help reduce that risk, Dr. Velander has jointly with the American Red Cross Holland Laboratory pioneered genetically engineered versions of human anticoagulant Protein C, human anti-hemophiliac factors VIII and IX, and fibrinogen (for fibrin glue precursor for hemostatic devices, tissue reconstruction and site specific drug delivery) from the milk of transgenic livestock. Dr. Velander's group also helped in pioneering efforts to humanize pig tissue to provide stop-gap alternatives for organ transplants.

His work on using this technology to promote hemophilia treatment in the developing countries of Latin America is currently embraced by the World Federation of Hemophilia (WFH), a United Nations NGO. He has had a three year collaboration on the treatment of hemophilia in Cuba with the Instituto de Hematologia y Inmunologia Habana Cuba that was licensed by the Department of Treasury. Dr. Velander has recently started a collaboration with the Foundation Oswaldo FioCruz of the Brasilian Ministry of Health to begin the modernization of hemophilia therapy using recombinant factors.

Dr. Velander's work on transgenic animals is known worldwide in scientific literature such Proceedings of the National Academy of Science and Nature Biotechnology as well as through international media including CNN's Future Watch, RAI's Quark, The New York Times, The Wall Street Journal, The London Daily Telegraph, the cover story of October 1999 National Geographic, May 1998 Discover, July 1998 Smithsonian and the January 1997 issue of Scientific American. His work with hemophilia factors was featured on 21st Century Medicine: Genetic Promises on the Discovery Health Channel in November 2001 and also in the exhibit "Engineering Genes" at the Museum of Science and Industry in Chicago, Illinois. Dr. Velander was recently inducted as a fellow of the American Institute of Medical and Bioengineering at a ceremony at the National Academy of Science in Washington, DC.

Dr. Velander was instrumental in the formulation of federal regulatory guidelines for human therapeutics derived from transgenic animals through his consultancy with the USFDA. He is a co-inventor of several US patents concerning gene transfer and the production of recombinant proteins of haemostasis whereupon all have been licensed and are in the process of commercialization. He continues to research genetically engineered versions of potent anticoagulants such as Protein C and Tissue Factor Pathway Inhibitor-Factor X chimera as well as Factor VIII and IX.

Education:

1987 Ph.D. in Chemical Engineering from The Pennsylvania State University, State College, Pennsylvania.

1980 M.ChE in Chemical Engineering from Illinois Institute of Technology, Chicago, Illinois.

1977 B.S. degree in Biochemistry from Illinois Benedictine College, Lisle, Illinois.

Professional Experience:

2004	D.R. Voelte Jr. and N.A. Keegan Endowed Chair in Engineering
2003	Chair, Department of Chemical Engineering, University of Nebraska, Lincoln
2003	Advisory Board Member Centre for Blood Research, University of British Columbia
2002	Faculty member School of Medical and Bioengineering VPISU
2002	Distinguished Lecturer, University of Mayaguez, Puerto Rico, *Merck Sharp & Dohme Lecture Series*
2001	Chief Technology Officer of ProGenetics-HemoCare LLC.
2000	Elected Fellow of the American Institute for Medical and Bioengineering
1998	W. Martin Johnson Professor of Engineering, VPISU.
1997	Full Professor, Department Chemical Engineering, VPISU
1994	Virginia Tech Alumni Award for Excellence in Research
1994	(June-November) Visiting Scientist: Calcium-dependent Monoclonal Antibody-Protein C Interactions using Biacore at Pharmacia Biotechnology AB, Uppsala, Sweden.
1992-1996	Associate Professor, Department Chemical Engineering, VPISU.
1986-1992	Assistant Professor, Department Chemical Engineering, VPISU.
1980-1982	Research Engineer: Drug Synthesis and Scale-up, Merck, Sharp and Dohme Research Laboratories, Merck and Co. Rahway, NJ.
1976-1977	Clinical Chemistry Technologist, Northwest Suburban Hospital, Arlington Heights, Illinois

Professional Memberships:

American Institute of Medical and Bioengineering
American Chemical Society

Invited talks Selected from the Last Five Years:

"Production of Biopharmaceuticals in the Milk of Pigs with Emphasis on Factor IX for Treatment of Hemophiliacs" in Emerging Trends in Genomics: Application and Approaches, "Animal Genomics Symposium 2003" October 9 & 10, 2003, Charles Hamner Conference Center, NC Biotechnology Center, 15 T.W. Alexander Drive, Research Triangle Park, NC

"Production of Factor IX in Transgenic Animals", National Hemophilia Foundation Workshop on Gene Therapy April 26, 2003, The Salk Institute, La Jolla, CA

"Transgenic Animals: Present Status of Technology and Future Applications" Biotechnology in the Barnyard, sponsored by PEW Foundation and USFDA, September 24, 2002, Dallas, Texas

"Recombinant Factor IX production in the milk of transgenic pigs: a potentially abundant, economical and safe source of type Haemophiliac therapy", Gene Therapy Symposia in the XXV International Congress of the WFH, May 19-24, 2002. Seville Spain.

"Production of Hemophilia Factors in Transgenic Animals", National Hemophilia Foundation Workshop on Gene Therapy April 2002, Philadelphia, PA

"Use of Transgenic Technologies to Meet the Hemophilia Needs of Less Developed Countries", Blood Plasma Production and Coagulation Factors Symposia in Biological Safety and Production 2001 Meeting, April 5, 2001, Vienna Virginia

"A Potential Treatment for Type B Hemophilia in Developing Countries Using the Milk of Transgenic Pigs", Hematologia 2001, May 16, 2001, Habana, Cuba

"Production of Factor VIII and Factor IX By Use of Transgenic Animals", XIVth Van Creveld Symposium, October 12th 2000, Universar Medisch Centrum, Utrecht, The Netherlands.

"Transgenic Production of Hemophiliac Factors" The XXIV World Congress of the World Federation for Hemophilia, Montreal, Canada July 16-21, 2000.

"Applications and Recombinant Sources of Fibrin Glue; Parallel Paths for Fibrin Sealant Research and Development", Cardiovascular Application of Fibrin Sealant Workshop, sponsored by the USFDA, DVM Laurel MD, May 8, 2000.

"Current Progress in the Production of Recombinant Human Fibrinogen in the Milk of Transgenic Animals", State of the Art Lecturer, XVI th Congress of the International Society on Thrombosis and Haemostasis, June 1997, Florence, Italy

Patents in Last Five Years:

Transgenic non-human mammals expressing human coagulation factor VIII and von Willebrand factor US 6,518,482 issued February 11, 2003

Expression of Active Human Factor IX in Mammary Tissue of Transgenic Animals US 09/367,087 issued February 5, 2002

Transgenic Fibrinogen CA 2183546 issued August 21 2001

Transgenic non-human mammals expressing human coagulation ***factor VIII*** and von Willebrand factor US 6,255,554 issued July 3, 2001

Transgenic Animal Expressing Human Coagulation Factor VIII and Von Willebrand Factor US 08/308,518 issued March 9, 1999

Expression of Active Human Protein C in the Mammary Tissue of Transgenic Animals Using A Long WAP Promoter US07/943246 issued November 1998.

Expression of Active Human Protein C in the Mammary Tissue of Transgenic Animals US08/247 issued December 1996.

Selected Publications in Last Ten Years:

S. P. Butler, T. K. O'Sickey, S.T. Lord, H. Lubon, F.C. Gwazdauskas, and W.H. Velander, "Secretion of Recombinant Human Fibrinogen by the Murine Mammary Gland", *Transgenic Research, (accepted May 5, 2004).*

M. Lindsay, G. C. Gil, A. Cadiz, C. Zhang, W. H. Velander, K. E. Van Cott. Purification of Recombinant DNA-derived Factor IX and Fractionation of Active and Inactive Subpopulations. *Journal of Chromatography*, 1026:149-157.2004

LC Bolling, RS Pleasant, SP Butler, WH Velander and FC Gwazdauskas (2003) An Evaluation of Sperm Mediated Gene Transfer, Transgenics 4: 77-86.

CA Schomotzer, SP Butler, RE Pearson, WH Velander, and FC Gwazdauskas (2003) Assessment of DNA Expression Following Cytoplasmic Microinjection of Condensed DNA into Murine Embryos Using Electropulsation, Transgenics, 4:55-63.

William Velander and Kevin Van Cott, Protein Expression Using Transgenic Animals, in *Handbook of Industrial Cell Culture Mammalian, Microbial, and Plant Cells* eds. Vinci, Victor A. and Parekh, Sarad R. , Human Press, Totawa NJ (2002).

Van Cott KE, Lubon H, Gwazdauskas FC, Knight J, Drohan WN, Velander WH. (2001). Recombinant Human Protein C Expression in the Milk of Transgenic Pigs and the Effect on Endogenous Milk Immunoglobulin and Transferrin Levels. *Transgenic Research*, 10: 43-51.

Van Cott K, Butler SP, Russell CG, Subramanian A, Lubon H, Gwazdauskas FC, Knight J, Drohan WN, and Velander WH. (1999) Transgenic pigs as bioreactors: a comparison of gamma-carboxylation of glutamic acid in recombinant human protein C and factor IX by the mammary gland. *Genet Anal* 15(35):15560

Van Cott K, and Velander WH. (1998) Transgenic animals as drug factories: a new source of recombinant protein therapeutics. *Expert Opinion on Investigational Drugs*, 7(10): 1683-1690.

A. Degener, M. Belew and W. H. Velander, AZn^{2+}-selective Purification of Recombinant Proteins from the Milk of Transgenic Animals, Journal of Chromatography A, 799:125-137, 1998.

T. Morcöl, A. Subramanian and W. H. Velander, ADot-Blot Analysis of the Degree of Covalent Modification of Proteins and Antibodies at Amino Groups,@ Journal of Immunological Methods, 203:45-53, 1997.

S. P. Butler, K. van Cott, A. Subramanian, F.C. Gwazdauskas and W. H. Velander, ACurrent Progress in the Production of Recombinant Human Fibrinogen in the Milk of Transgenic Animals@ Thrombosis and Haemostasis, 78, 1:537-542, 1997.

R. K. Paleyanda, W. H. Velander, T. K. Lee, D. H. Scandella, F. C. Gwazdauskas, J. W. Knight, L. W. Hoyer, W. N. Drohan and H. Lubon, ATransgenic Pigs Produce Functional Human Factor VIII in Milk,@ Nature Biotechnology, 15:971-975, 1997.

W. H. Velander, H. Lubon, and W. Drohan, "Transgenic Livestock as Drug Factories," Scientific American, Vol. 276, No. 1, pp. 54-58, January 1997.

K. E. Van Cott, H. Lubon, C. G. Russell, S. P. Butler, F. C. Gwazdauskas, J. Knight, W. N. Drohan and W. H. Velander, "Phenotypic and Genotypic Stability of Multiple Lines of Transgenic Pigs Expressing Recombinant Human Protein C," Transgenic Research, 6:1-10, 1997.

K. E. Van Cott, B. L. Williams, F. Gwazdauskas, T. Lee, H. Lubon, W. N. Drohan and W. H. Velander, "Affinity Purification of Biologically Active and Inactive Forms of Recombinant Human Protein C Produced in the Porcine Mammary Gland," Journal of Molecular Recognition, Vol. 9, Issue 6, pp. 407-414, 1997.

A. Subramanian, R. K. Paleyanda, H. Lubon, B. L. Williams, F. C. Gwazdauskas, J. W. Knight, W. N. Drohan and W. H. Velander, ARate Limitations in Posttranslational Processing by the Mammary Gland of Transgenic Animals,@ The Annals of the New York Academy of Sciences, 782, 87-96, 1996.

H. Lubon, R. K. Paleyanda, W. H. Velander and W. N. Drohan, ABlood Proteins from Transgenic Animal Bioreactors,@ Transfusion Medicine Reviews, 10:131-143, 1996.

T. K. Lee, N. Bangalore, W. Velander, W. N. Drohan and H. Lubon, AActivation of Recombinant Human Protein C,@ Thrombosis Research, 82:225-234, 1996.

A. Subramanian and W.H. Velander, AThe Effect of Antibody Orientation of Immunosorbent Performance,@ Journal of Molecular Recognition, 9:528-535 (1996).

R. L. Page, R. S. Canseco, C. G. Russell, J. L. Johnson, W. H. Velander and F. C. Gwazdauskas, ATransgene Detection During Early Murine Embryonic Development After Pronuclear Microinjection,@ Transgenic Research, 4:12-17, 1995.

R.L. Page, S.P. Butler, A. Subramanian, F.C. Gwazdauskas, J.L. Johnson, and W.H. Velander, ATransgenesis in Mice by Cytoplasmic Injection of Polylysine/DNA Mixtures,@ Transgenic Research, 4:353-360, 1995.

T. Morcol, R.M. Akers, J.L. Johnson, B.L. Williams, F.C. Gwazdauskas, J.W. Knight, H. Lubon, R.K. Paleyanda, W.N. Drohan and W.H. Velander, "The Porcine Mammary Gland as a Bioreactor for Complex Proteins," The Annals of the New York Academy of Sciences, 721, 218-233, 1994.

R. L. Krisher, J. R. Gibbons, R. S. Canseco, J. L. Johnson, C. G. Russell, D. R. Notter, W. H. Velander and F. C. Gwazdauskas, AInfluence of Time of Gene Microinjection on Development and DNA Detection Frequency in Bovine Embryos,@ Transgenic Research, 3:226-231, 1994.

R. L. Krisher, F. C. Gwazdauskas, R. L. Page, C. G. Russell, R. S. Canseco, A. E. T. Sparks, W. H. Velander, J. L. Johnson and R. E. Pearson, AOvulation Rate, Zygote Recovery and Follicular Populations in FSH-Superovulated Goats Treated with $PGF_{2\alpha}$ and/or GnRH, Theriogenology, 41:491-498, 1994.

A.E.T. Sparks, R. S. Canseco, C. G. Russell, J. L. Johnson, J. L. Page, W. H. Velander, M. L. McGilliard, and F. C. Gwazdauskas, ADevelopment of Bovine Morulae After Bisection and Biopsy and Assessment of DNA Amplification by the Polymerase Chain Reaction,@ Animal Reproduction Science, 35:1-7, 1994.

A.E.T. Sparks, R.S. Canseco, C.G. Russell, J.L. Johnson, H.D. Moll, W.H. Velander, and F.C. Gwazdauskas, "Effects of Time of Deoxyribonucleic Acid Microinjection on Gene Detection and In Vitro Development of Bovine Embryos," Journal of Dairy Science, 77:718-724, 1994.

M.A. Hajdu, J.W. Knight, R.S. Canseco, R.L. Krisher, W.H. Velander, R.E. Pearson and F.C. Gwazdauskas, "Effect of Culture Conditions, Donor Age, and Injection Site on In Vitro Development of DNA Microinjected Porcine Zygotes," Journal of Animal Science, 72:1299-1305, 1994.

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A. Subramanian, K.E. Van Cott, D.S. Milbrath and W.H. Velander, "Role of local antibody density effects on immunosorbent efficiency," Journal of Chromatography A 672:11-24, 1994.

W.L. Fodor, B.L. Williams, L.A. Matis, J.A. Madri, S.A. Rollins, J.W. Knight, W. Velander and S.P. Squinto, "Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic hyperacute organ rejection," Proc. Natl. Acad. Sci. USA, 91:11153-11157, 1994.

W.N. Drohan, D.-W. Zhang, R.K. Paleyanda, R. Chang, M. Wroble, W. Velander and H.K. Lubon, "Inefficient processing of human protein C in the mouse mammary gland," Transgenic Research, 3:355-364, 1994.

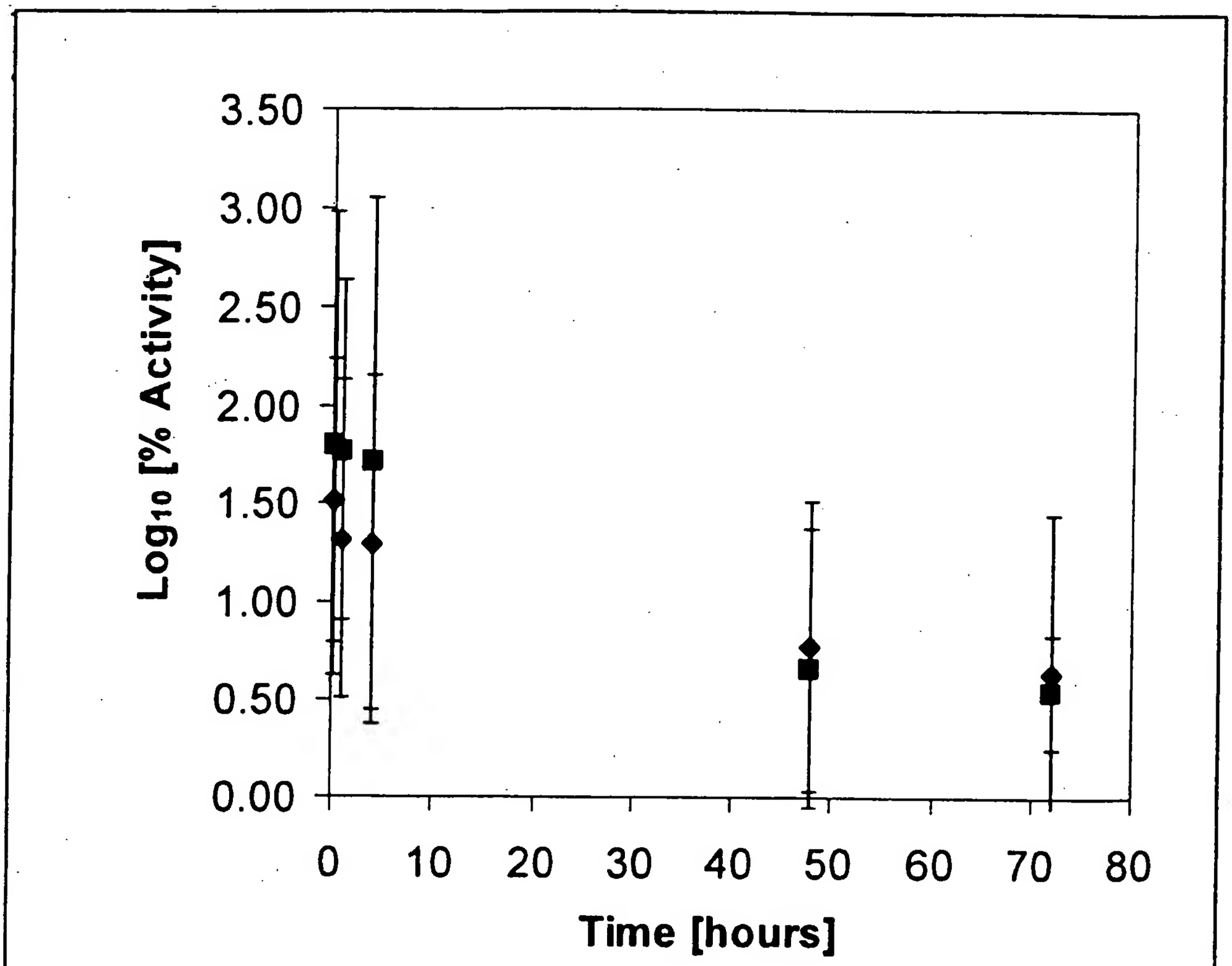

Figure 1. Semi-log plot of Factor IX activity measured plasma samples taken after intravenous injection of Mononine® (■, n = 8) and transgenic-derived Factor (♦, n = 8) in the Factor IX-knockout hemophilia B mouse model. Factor IX activity in collected mouse plasma was measured by the aPTT assay.